Characterization of Guinea Pig C-Type Virus[1] (37322)

P. A. GROSS, C. K. Y. FONG, AND G. D. HSIUNG

Veterans Administration Hospital, West Haven, Connecticut 06516; and Departments of Internal Medicine and Laboratory Medicine, Yale University School of Medicine, New Haven, Connecticut 06510

In 1967 Nadel *et al.* and Opler reported the observation of C-type virus particles in tissues and leukocytes of strain 2 guinea pigs with lymphoblastoid leukemia (1, 2). Subsequently, similar findings were reported by other investigators (3–5). The C-type virus, considered as a possible etiological agent for guinea pig leukemia, however, had not been cultivated *in vitro* until our recent study (6). The activation of guinea pig C-type virus in cultured spleen cells derived from leukemic animals was accomplished by maintaining cultures in a medium containing 5-bromo-2′-deoxyuridine (BUDR).

We also found that the C-type virus could be induced in cultured spleen or kidney cells prepared from normal strain 2 guinea pigs. In addition, a continuous spleen cell line derived from a leukemic guinea pig was found to yield C-type virus as long as the cultures were maintained in the medium containing BUDR. Thus, the availability of the guinea pig C-type virus preparations enabled us to further characterize this virus, and study some of its biophysical and biochemical properties. The results are included in the present report.

Materials and Methods. Cell cultures. Spleen or kidney tissues were removed aseptically from strain 2 or strain 2-Hartley hybrid guinea pigs with or without leukemia. Cell suspensions were prepared as those previously described (4, 6). The tissues were minced and dispersed by trypsin, 0.25%. After centrifugation, the spleen cells were resuspended in medium RPMI-1640 at 1:1000 (v/v) and the kidney cells were resuspended in HK growth medium at 1:400 (v/v). Both cultures were maintained in basal medium Eagle's (BME) containing 5 or 10% fetal bovine serum. In addition, a continuous spleen cell line (LGPS) derived from a leukemic guinea pig was also used.

Induction of C-type virus. Methods of induction of C-type virus were essentially the same as those previously reported (6). Both primary cell cultures or the continuous LGPS cell line were maintained in the BME containing 40 μg/ml of BUDR for various periods of time in order to obtain sufficient quantities of C-type virus for the studies.

Virus nucleic acid determination. ^{3}H-Uridine, 25 μCi/ml in BME without serum was added to either the primary cultures or the LGPS cell line that had been treated with BUDR for 4 or 7 days, respectively. The tissue culture fluid was harvested 4 days later and centrifuged at 2000*g* for 30 min at 4° to remove the cell debris. The virus was pelleted at 95,000*g* for 90 min at 4°. Virus pellets were layered onto a 24 ml 15–65% sucrose gradient in TEN buffer (0.01 *M* Tris HCl, 0.001 *M* EDTA, 0.1 *M* NaCl, pH 7.2) and centrifuged at 95,000*g* for 3 hr at 4° on a SW 27 rotor. A light scattering band was evident at a density of 1.15 g/ml. The band was collected, diluted and layered onto another 15–65% sucrose gradient and centrifuged again for 3 hr at 95,000*g*. The band from the second sucrose gradient was diluted, layered onto a 15–65% sucrose gradient and spun to equilibrium for 16–20 hr at 95,000*g*. All gradients were collected by puncturing a

[1] This investigation was supported in part by research funds from the Veterans Administration and Public Health Service Research Grant AI-08648 from the National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD.

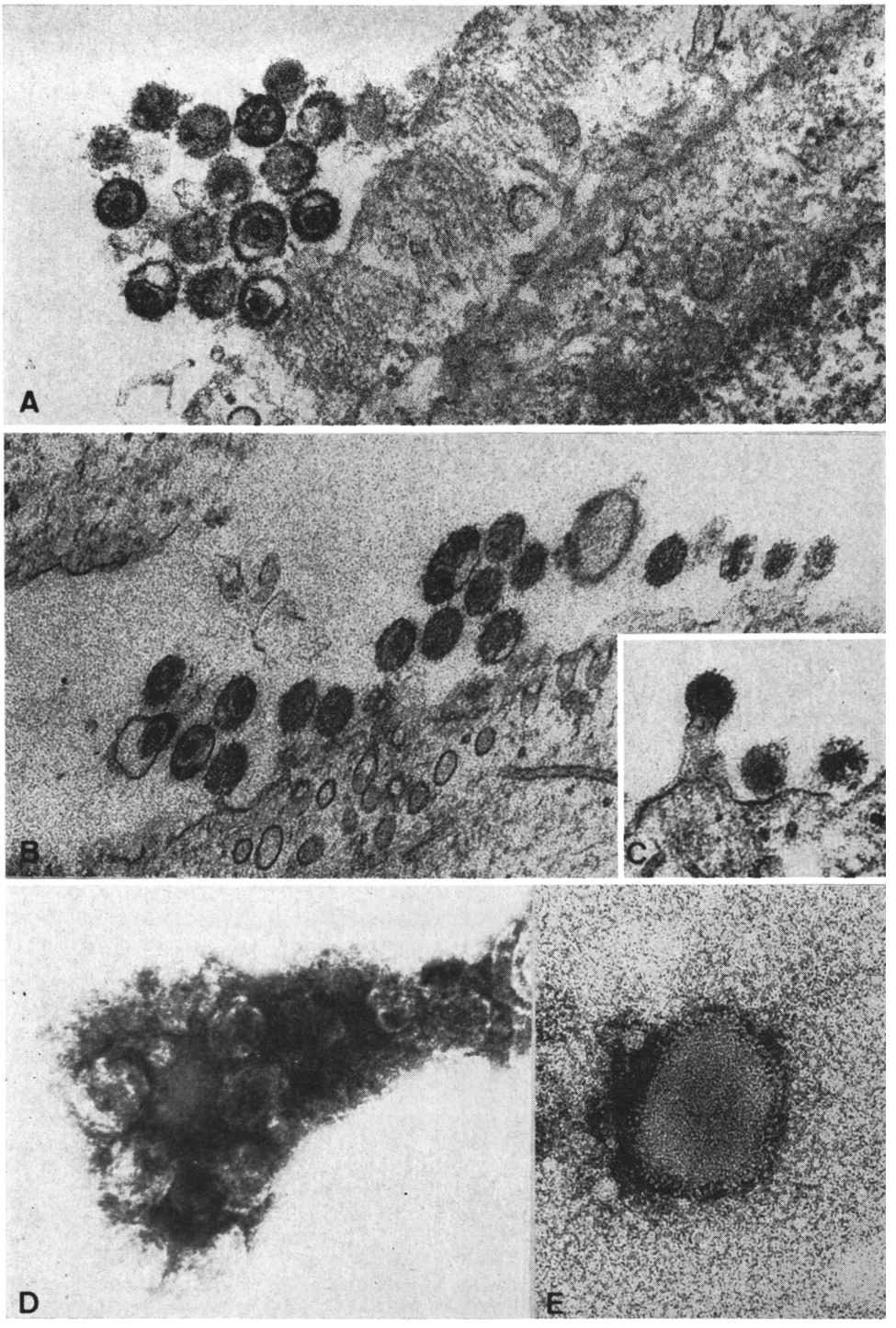

FIG. 1. Electron micrographs of the guinea pig C-type virus. (A) A spleen cell derived from a normal hybrid guinea pig AD007, 17 days in culture, and 4 days after BUDR treatment, ×64,800. (B) A cell in the LGPS cell line derived from a leukemic guinea pig No. 325, passage No. 11, 13 days after BUDR treatment, ×64,800. (C) A C-type virus budding from the cell surface of a primary spleen cell of guinea pig No. 325, 2 days after BUDR treatment, ×64,800. (D) Electron micrograph of an aggregate of guinea pig C-type virus, fixed with 2% glutaraldehyde and stained with phosphotungstic acid (PTA) at pH 7.0 ×100,800. (E) A single virus particle stained with PTA alone showing surface projections. ×126,000.

hole in the bottom of the centrifuge tube. Acid-insoluble counts were determined on each gradient fraction.

Electron microscopy. Cells from monolayers of primary cultures or LGPS cell line treated or untreated with BUDR were fixed in 2% glutaraldehyde *in situ.* Cell pellets were postfixed with osmium tetroxide and embedded in Epon as described separately (7). Thin sections were stained with uranyl acetate and lead citrate and examined with a Philips EM 300 electron microscope. For negative staining, purified virus suspensions were added to formvar coated grids which were allowed to dry at room temperature. The grids containing virus were then either fixed with 2% glutaraldehyde followed by staining with 1% phosphotungstic acid (PTA) at pH 7.0, or stained with PTA without prior fixation. All the specimens prepared for negative staining were examined immediately after staining.

Results. Morphology of the virus. Numerous C-type virus particles, 110 nm in diameter, were observed in primary spleen or kidney cells derived from normal strain 2 or hybrid guinea pigs when cell cultures were treated with BUDR for 3–4 days (Fig. 1A). Similar virus particles were found in the LGPS cell line treated with BUDR for 7 days or longer (Fig. 1B). It was noted that the highest numbers of C-type virus were observed in primary cultures which were treated with BUDR for 4 days, whereas 7–14 days were necessary for the LGPS line. Most of the mature C-type virus particles were found in the extracellular space. Intracellular virus particles were seen in the cytoplasmic vacuoles. Virus particles budding at the cell surface were observed occasionally (Fig. 1C). C-type virus was rarely found in parallel cultures that were not treated with BUDR. Monolayer cultures of primary or LGPS cells failed to show any evidence of hemadsorption when treated with guinea pig red blood cell suspension.

In glutaraldehyde fixed, PTA stained specimens of purified virus, particles appeared spherical and uniform in size with an average diameter 105 nm (Fig. 1D). In samples stained with PTA alone without prior fixation, virus particles were more pleomorphic with a size ranging from 120 to 150 nm, and surface projections were often observed (Fig. 1E). The internal structure of nucleocapsid cores, 40A strands, was observed in some disrupted virions.

Nucleic acid type. When the BUDR treated cultures either obtained from primary spleen or kidney cells or from LGPS cell line were labeled with ^{3}H-uridine, the peak of radioactivity was found to be associated with a visible band containing the virus particles as shown in Fig. 1D. The distribution of the radioactivity obtained in the final equilibrium sucrose gradient of the purified ^{3}H-uridine labeled virus is illustrated in Fig. 2. It can be seen that the peak of uridine label occurred at a density of 1.17 ± 0.01 g/ml. When ^{3}H-thymidine was used as the radioactive label, radioactivity was not detectable in the final gradient. When the ^{3}H-uridine labeled virus nucleic acid was extracted with ether and treated with pancreatic ribonuclease, acid precipitable radioactivity was markedly reduced. Attempts have been made to detect a RNA-dependent DNA polymerase in the concentrated C-type virus preparations. However, all samples have

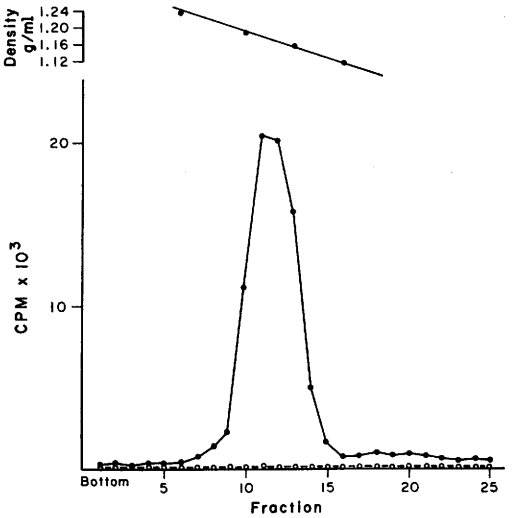

FIG. 2. Sucrose density-gradient analysis of purified ^{3}H-labeled guinea pig C-type virus [^{3}H-uridine (●—) and ^{3}H-thymidine (○—)]. The ^{3}H-uridine peak at 1.17 ± 0.01 g/ml coincided with a light scattering band.

failed to show the presence of the enzyme, and GS-3 antigen has not been found (Gilden, personal communication).

Discussion. Data obtained from the present study indicate that the guinea pig C-type virus resembles other members of the C-type viruses (8). It is an RNA containing virus with a density in sucrose of 1.17 ± 0.01 g/ml. Morphologically, both in purified preparations and infected cells, typical structures of C-type viruses were seen. The possibility of the presence of a morphologically similar paramyxovirus was eliminated since guinea pig RBC did not adsorb onto the C-type virus yielding monolayer cultures.

However, the absence of RNA-dependent DNA polymerase and the GS-3 antigen in the guinea pig C-type virus preparations hampered our final identification since these properties were essential for all mammalian C-type viruses previously described (9). It is possible that the concentration of this enzyme in our C-type virus preparations was not high enough for the detection method. Similarly, a more concentrated virus stock may be necessary for the detection of the GS-3 antigen. Whether or not the guinea pig C-type virus is actually lacking in this group specific GS-3 antigen is currently under investigation.

The induction of guinea pig C-type virus in cultured cells derived from leukemic and nonleukemic strain 2 or hybrid guinea pigs was similar to those described for the activation of murine or human C-type viruses (10–12). The finding of C-type virus in normal healthy guinea pigs was of particular interest. Prior to our findings, C-type virus was observed only in tissues and leukocytes of leukemic guinea pigs, but not in healthy control animals (3). Huebner and Todaro (13) postulated that C-type RNA viruses probably are present in cells of all mammalian species. If such is the case, then the presence of the C-type virus in strain 2 guinea pigs would suggest that this virus is the oncogene in this animal species, since strain 2 guinea pigs are the susceptible hosts for the guinea pig leukemia (1–5). However, the regular presence of a herpes-like virus in strain 2 guinea pigs (4) might also be included in this hypothesis. At the moment, neither virus alone has been capable of inducing leukemia in guinea pigs (14). Further studies of both guinea pig C-type virus and guinea pig herpesvirus in relation to neoplastic disease in this animal species are currently in progress.

Summary. A guinea pig C-type virus was successfully isolated from primary and passaged cells derived from leukemic and normal strain 2, and strain 2-Hartley hybrid guinea pigs. Activation of the virus required BUDR treatment. Morphologically, both in infected cells and in negatively stained preparations, the guinea pig C-type virus resembles other members of the C-type virus group. Purified guinea pig C-type virus labeled with ^{3}H-uridine had an equilibrium density of 1.17 ± 0.01 g/ml in sucrose.

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Received Feb. 9, 1973. P.S.E.B.M., 1973, Vol. 143,